1. Phylogeny  
   MYLK3, also known as cardiac MLCK (cMLCK) or Cardiac‐MyBP-C-associated Ca/CaM kinase, is a member of the myosin light chain kinase family. It is distinct from the smooth muscle MLCK (encoded by MYLK1) and skeletal muscle MLCK (encoded by MYLK2), sharing a conserved catalytic domain with approximately 58% identity to the skeletal isoform and 44% to the smooth isoform. MYLK3 is expressed exclusively in cardiac tissue, with orthologs documented in mouse, rat, and human, and it is part of the evolutionary core set of MLCK enzymes that emerged early in eukaryotic evolution (chan2008identificationofcardiacspecific pages 1-2, tsukamoto2013biochemicalandphysiological pages 2-5, sutter2004orthologousrelationshipof pages 1-2).
2. Reaction Catalyzed  
   MYLK3 catalyzes the ATP-dependent phosphorylation of myosin regulatory light chains. The chemical reaction it facilitates can be generalized as follows:  
     ATP + [myosin regulatory light chain]-(L‑serine) → ADP + [myosin regulatory light chain]-(L‑serine phosphate) + H⁺.  
   In cardiac cells, MYLK3 preferentially phosphorylates MYL2, the regulatory light chain isoform in cardiomyocytes, primarily at a conserved serine residue (Ser15 in human cardiac muscle) (chan2008identificationofcardiacspecific pages 8-9, chang2016roleofmyosin pages 1-3, tsukamoto2013biochemicalandphysiological pages 1-2).
3. Cofactor Requirements  
   The catalytic activity of MYLK3 requires divalent metal ions, with Mg²⁺ serving as an obligatory cofactor for nucleotide binding during the phosphorylation reaction. In addition, although its activity is described as constitutive, a high affinity for Ca²⁺/calmodulin complexes has been noted, and several reports indicate that binding of Ca²⁺/calmodulin can stimulate its kinase activity by approximately two‐fold. The cellular regulation of MYLK3 is thus linked to intracellular Ca²⁺ levels, even though it may exhibit basal activity in vitro (tsukamoto2013biochemicalandphysiological pages 2-5, chang2016roleofmyosin pages 4-6).
4. Substrate Specificity  
   MYLK3 exhibits strict substrate specificity toward the cardiac isoforms of the myosin regulatory light chain. It phosphorylates the ventricular (MLC2v) and atrial (MLC2a) isoforms, targeting a key serine residue—Ser15 in human cardiac tissue—for phosphorylation. The substrate recognition involves flanking sequences that are rich in basic and hydrophobic residues, which are typical features of the MLCK consensus recognition motif. This specificity ensures proper modulation of myosin ATPase activity and contractile function exclusively in cardiac myocytes (chan2008identificationofcardiacspecific pages 8-9, stull2011myosinlightchain pages 4-5).
5. Structure  
   MYLK3 is approximately 86–90 kDa in size and is organized into distinct domains. It contains a unique amino‐terminal region that lacks homology to comparable segments in smooth or skeletal MLCKs, conferring cardiac-specific functionality. The carboxyl-terminal portion harbors the conserved catalytic (kinase) domain which is responsible for transferring phosphate groups from ATP to the target serine residue on myosin regulatory light chains. In addition to the kinase domain, MYLK3 is believed to contain a regulatory segment, which in other MLCK family members mediates calmodulin binding; however, the distinctive structure of the cardiac isoform suggests deviations in domain organization and potentially in regulatory mechanisms. The catalytic domain features the canonical features of serine/threonine kinases, including an activation loop, a C-helix, and elements forming the hydrophobic spine necessary for efficient catalysis (chan2008identificationofcardiacspecific pages 1-2, chan2008identificationofcardiacspecific pages 3-4, tsukamoto2013biochemicalandphysiological pages 2-5).
6. Regulation  
   MYLK3 is subject to multiple regulatory mechanisms. It is phosphorylated, and its phosphorylation levels are increased in response to adrenergic stimuli (for example, phenylephrine), which correlates with enhanced phosphorylation of the regulatory light chain in ventricular myocytes. Although its activity appears to be constitutive in basal conditions, binding of Ca²⁺/calmodulin has been shown to stimulate its catalytic output by approximately two‐fold. Furthermore, regulatory modifications such as phosphorylation at specific serine/threonine sites in its sequence may modulate its interactions with substrates and localization within the cell. In addition, differential expression levels in neonatal versus adult cardiac tissue and under pathological conditions (such as heart failure or pressure overload) indicate regulation at the transcriptional or post-translational level within the context of cardiac remodeling (chan2008identificationofcardiacspecific pages 1-2, tsukamoto2013biochemicalandphysiological pages 6-7, chang2016roleofmyosin pages 3-4).
7. Function  
   MYLK3 plays a critical role in cardiac physiology by phosphorylating the myosin regulatory light chain (MYL2) in cardiomyocytes. This phosphorylation event is essential for proper sarcomere formation and organization, contributing to baseline myosin ATPase activity and contractile function. In experimental assays, both overexpression and knockdown of MYLK3 in cultured cardiomyocytes have demonstrated its importance in modulating contractility and maintaining proper sarcomere assembly. MYLK3 expression is specific to cardiac muscle – being present in both atria and ventricles – and its kinase activity is integral to establishing the approximately 40% baseline phosphorylation of myosin regulatory light chain required for normal contractile performance (chan2008identificationofcardiacspecific pages 1-2, chan2008identificationofcardiacspecific pages 3-4, chang2016roleofmyosin pages 1-3, tsukamoto2013biochemicalandphysiological pages 2-5).
8. Other Comments  
   MYLK3 is alternatively known as Cardiac-MyBP-C-associated Ca/CaM kinase. Although specific small-molecule inhibitors targeting MYLK3 have not been detailed in the provided literature, the kinase’s critical role in maintaining basal phosphorylation of the myosin regulatory light chain has been highlighted in the context of cardiac contractility and sarcomere integrity. Alterations in MYLK3 expression or activity are associated with cardiac pathological conditions, including heart failure and pressure overload, suggesting that it may serve as a potential therapeutic target. No disease mutations or direct inhibitor profiles have been reported within the scope of the current references (chan2008identificationofcardiacspecific pages 1-2, tsukamoto2013biochemicalandphysiological pages 6-7, chang2016roleofmyosin pages 18-23).
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